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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53 (c).

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TITLE OF THE INVENTION (280 characters max)

NOVEL THERAPEUTIC USE

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

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Respectfully submitted,

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NOVEL THERAPEUTIC USE

FIELD OF INVENTION

The present invention relates to the use of certain indolinone compounds in the prevention, treatment or amelioration of multiple sclerosis.

BACKGROUND OF THE INVENTION

Multiple sclerosis is an auto-immune inflammatory disease of the central nervous system characterised by T-cell infiltration, demyelination of white matter and axonal injury. The disease mostly affects young adults with an onset at 20-40 years of age and affects twice as many women as men (A. Compton and A. Coles, *The Lancet 359*, 6 April 2002, pp. 1221-1231). Multiple sclerosis is more common in temperate climate zones and thus has a prevalence of 50-130 out of 100,000 in northern Europe and North America (N. Hellings et al., *Immunologic Research 25*(1), 2002, pp. 27-51). The higher incidence and prevalence of multiple sclerosis in certain European populations has not been adequately explained, it is believed that increased genetic susceptibility in these populations is partly responsible. The presence of a genetic element in the etiology of the disease is supported by family studies showing that first-degree relatives of multiple sclerosis patients have a 20-40 times increased risk of developing the disease relative to the general population (J.H. Noseworthey et al., *New England Journal of Medicine 343*(13), 2000, pp. 938-952). Furthermore, it has been recognised that populations with a high frequency of for instance the HLA-DR2 allele have a significantly higher risk of developing multiple sclerosis (Hellings et al., *supra*; Noseworthy et al., *supra*). However, no single major susceptibility gene for multiple sclerosis has been identified so far, and the results of genome screens conducted to identify susceptibility genes rather point to multiple genes exerting a moderate effect (Hellings et al., *supra*).

Based on these studies, it would appear that genetic susceptibility is not enough in itself to provoke multiple sclerosis. This theory is given credence by the fact that the rate of prevalence of multiple sclerosis among people of European descent living outside Europe is half of that persisting in parts of northern Europe and that the low frequency of multiple sclerosis in Africans increases significantly among first-generation decendants living in Europe (Compton and Coles, *supra*). Environmental factors have therefore also been proposed as contributing to the development of multiple sclerosis. In particular, it is believed that certain antigens present on pathogenic organisms such as viral or bacterial epitopes which structurally resemble autoantigenic epitopes of, for instance, myelin basic

protein, proteolipid protein, myelin-associated glycoprotein or oligodendrocyte glycoprotein, which are all components of the myelin sheath, may lead to activation of T-cells that are reactive with such antigenic epitopes and initiating the inflammatory process eventually resulting in clinical manifestations of multiple sclerosis. This phenomenon is generally referred to as molecular mimicry (Hellings et al., *supra*; A. Bar-Or et al., *J. Neuroimmunol. 100*, 1999, pp. 252-259; A. Karni and H.L. Weiner, "Organ-Specific Inflammatory Diseases" Chapter 77 in *Clinical Immunology; Principles and* Practice, 2nd Ed. (R.R. Rich et al., Eds.), Mosby, London, 2001).

Multiple sclerosis is usually defined as either a relapsing-remitting or a progressive disease. The relapsing-remitting form with which 80% of the patients are initially afflicted (Compton and Coles, *supra*) is characterised by discrete attacks with full or partial recovery between relapses. In 40-50% of the patients, the disease eventually becomes progressive (secondary progressive stage). The disease may also be progressive from the outset (primary progressive form) characterised by a gradual decline in neurological function with no periods of remission. The clinical symptoms of the relapsing-remitting form of multiple sclerosis may vary widely from one patient to the other, but commonly affected individuals initially experience some degree of visual and sensory impairment, limb paresthesias, limb weakness, clumsiness, fatigue and gait ataxia, while in the later stages cognitive impairment, progressive quadriparesis, sensory loss, ataxic tremors, pain and spasticity are more common (Noseworthy et al., *supra*). The primary progressive form may initially manifest as one or more of these symptoms, gradually declining into quadriparesis, cognitive decline, visual loss, brainstem syndromes and cerebellar, bowel and bladder dysfunction (Noseworthy et al., *supra*).

Pathologically, multiple sclerosis is characterised by the presence of demyelinated plaques or sclerotic lesions where the myelin sheath surrounding the axons is destroyed. The inflammatory infiltrate in the lesions is composed of T-cells, B-cells, microglia and macrophages which interact with the myelin sheath and participate in the demyelinating process by local production of immune-related molecules such as adhesion molecules, cytokines and chemokines as well as demyelinating antibodies, oxygen free radicals and nitric oxide (Karni and Weiner, *supra*). While axonal destruction is not pronounced in the early stages of the disease (although more pronounced in patients suffering from the primary progressing form), demyelination of the axons results in slowing and blocking conductivity (Noseworthy et al., *supra*). Regression of the symptoms may be associated with partial remyelination after the initial inflammation has subsided showing that oligodendrocytes (myelin-producing cells) are present in the lesions (Karni and Weiner, *supra*). In later stages, irreversible axonal injury, gliotic scarring and gradual loss of

oligodendrocyte progenitor cells may result from repeated episodes of inflammatory attack and leads to permanent loss of neurological function (Noseworthy et al., *supra*).

While the immunopathogenesis of multiple sclerosis is still largely unknown, it has been shown that autoreactive T-cells specific for myelin basic protein and other antigens of the central nervous system exist in the periphery of healthy individuals as wells as individuals who later develop multiple sclerosis (Bar-Or et al., *supra*; O'Connor et al., *J. Clin. Immunol. 21*(2), 2001, pp. 81-93). Thus, the presence of myelin-reactive T-cells in the periphery is not enough in itself to explain the development of multiple sclerosis. In multiple sclerosis patients, these T-cells become activated, possibly by cross-reactivity with bacterial or viral antigens that structurally resemble myelin antigens (i.e. the phenomenon known as molecular mimicry) and/or by bacterial superantigens, and persist in an enhanced state of activation (Hellings et al., *supra*). It has been found that the autoreactive T-cells are predominantly CD4+ T helper cells type 1 (Th1) producing interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour necrosis factor (TNF-α) (B. Gran and A. Rostami, *Current Neurology and Neuroscience Reports 1*, 2001, pp. 263-270). In order for such proinflammatory T-cells to migrate to the central neurvous system, they express chemokine receptors, adhesion molecules and matrix metalloproteinases that enable them to cross the blood-brain barrier. Thus, it has been found that expression levels of the chemokines which are chemotactic for Th1 cells, IP-10 and RANTES, and their corresponding receptors, CXCR3 and CCR5, are elevated in sclerotic lesions and cerebrospinal fluid of multiple sclerosis patients (Bar-Or et al., *supra*). Altered levels of the adhesion molecules ICAM-1 and VCAM-1 have been identified on endothelial cells of multiple sclerosis lesions (O'Connor et al., *supra*). ICAM-1 and VCAM-1 are important for endothelial-leukocyte interactions and leukocyte extravasation. Matrix metalloproteinases expressed by activated T-cells, monocytes and astrocytes may disrupt the basement membrane of the blood-brain barrier and facilitate transmigration of T-cells and breakdown of the extracellular matrix (O'Connor et al., *supra*).

Once the T-cells have entered the central nervous system they become reactivated on encountering the autoantigen, e.g. myelin basic protein, presented by MHC class II expressing antigen presenting cells (microglia and dendritic cells), and the Th1 cells respond by producing proinflammatory cytokines such as TNF-α, IFN-γ and IL-2, while the Th2 cells produce anti-inflammatory cytokines such as IL-4, IL-5 and IL-10 (Bar-Or et al., *supra*). In turn, the inflammatory process leads to up-regulation of MHC class II expression and adhesion molecules on the blood-brain barrier endothelium, facilitating a further influx of T-cells, B-cells and macrophages and hence an amplification of the inflammatory response (Hellings et al., *supra*). This theory is supported by the finding that myelin basic protein reactive T-cell clones from multiple sclerosis patients were found to secrete increased

amounts of different cytokines such as TNF-α, IL-2 and IL-10 (Hellings et al., *supra*). Demyelination (myelin destruction) is believed to be brought about by the combined effects of cytotoxic cells (macrophages and T-cells), oxygen free radicals, demyelinating autoantibodies and cytokine-induced toxicity (Hellings et al., *supra*).

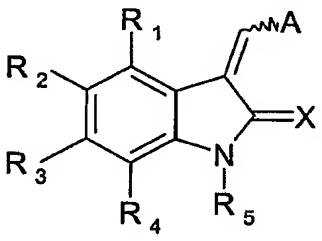
Traditionally, corticosteroids such as prednisolone have been administered intravenously to multiple sclerosis patients during acute relapses in order to attenuate the inflammatory response. It has been found that treatment with corticosteroids during relapses reduces the duration of relapses and their short-term morbidity, but not the permanent disabilities resulting from repeated relapses (Compton and Coles, *supra*). Furthermore, treatment with potent corticosteroids at high doses has serious side effects, notably osteoporosis, aseptic bone necrosis, skin atrophy, striae cutis, insomnia, myopathy, posterior and capsular cataract and glaucoma as well as reactivation of the disease upon cessation of treatment. More recently, interferon-β (IFN-β) has been introduced as a treatment of relapsing-remitting multiple sclerosis and has been found to decrease the rate of relapse, increase the proportion of patients who were relapse free and reduce the number of patients who had moderate to severe relapses. On the other hand, INF-β treatment is extremely costly and its long-term efficacy has not been established. There is concern that the treatment may induce the formation of neutralising antibodies that may reduce the activity of IFN-β (Noseworthy et al., *supra*). Most of the patients initially experience flu-like symptoms when treated with IFN-β. Glatiramer acetate is another recent treatment based on a mixture of random synthetic peptides intended to mimic myelin basic protein. In a double-blind trial of relapsing-remitting multiple sclerosis, glatiramer acetate was found to decrease the rate of relapse. Glatiramer acetate is believed to be most effective for mildly disabled patients with a recent diagnosis of multiple sclerosis. Fewer treatment options exist for patients in the progressive phase of the disease. Immunosuppressive therapy, e.g. with cyclophosphamide or methotrexate, is frequently attempted, but it is generally recognised that once the disease enters the progressive stage treatment is very difficult. IFN-β has been in clinical trials for secondary progressive multiple sclerosis but the results did not show that the treatment slowed progression of disability and the benefits of this treatment in secondary progressive disease are controversial.

Thus, it would appear that there is a continued medical need for effective treatment of multiple sclerosis. It would also be beneficial if a medicament suitable for oral administration were to be developed.

SUMMARY OF THE INVENTION

In the course of research leading to the present invention, it was surprisingly found that certain indolinone compounds which have previously been shown to be inhibitors of receptor tyrosine kinases and suggested for the treatment of cancer exhibit a substantial level of activity in experimentally induced autoimmune encephalomyelitis (EAE) which is generally recognised as an animal model of multiple sclerosis. EAE may be induced by injection of antigenic peptides of myelin such as myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein. EAE is an inflammatory condition of the central nervous system characterised by T-cell infiltration and focal demyelination. EAE can also be induced by transfer of myelin reactive T-cells to normal individuals.

Accordingly, the present invention relates to the use of a compound of general formula I



I

wherein

X is O or S;

R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

A is phenyl or a monocyclic or bicyclic heteroaryl ring selected from the group consisting of pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, tetrazole and indole, optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n

are as indicated above, the zigzag line indicating that the group denoted A is present as the E- or Z-isomer;
R_5 is hydrogen or alkyl;
or pharmaceutically acceptable salts thereof, for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay of the onset of or reduce the relapse rate in multiple sclerosis.

In another aspect, the invention relates to a method of preventing, treating or ameliorating multiple sclerosis, or delaying the onset of or reducing the relapse rate in multiple sclerosis, the method comprising administering, to a patient in need thereof, a pharmacologically effective amount of a compound of general formula I as shown above.

Compounds of formula I are disclosed in WO 96/40116 in which they are indicated to be inhibitors of tyrosine kinases and as such useful in the treatment of cancer, blood vessel proliferative disorders, fibrotic disorders, mesangial cell proliferative disorders and metabolic diseases. There is no suggestion that compounds within this group might have any utility in the treatment of multiple sclerosis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of EAE with compound A. Mice were immunized on day 0 with the $PLP_{139-153}$ peptide. Compound A were dosed daily i.p. from day 0 as follows; compound A-50 (50 mg/kg); compound A-25 (25 mg/kg); compound A-10 (10 mg/kg); compound A-4 (4 mg/kg). Control groups were given either suspension vehicle i.p. from day 0 or dexamethasone (1 mg/kg) p.o. from day 1. The experiment was terminated on day 21 p.i. **A)** The frequency of animals with EAE symptoms. Mice dead or sacrificed during the experiment are included. **B)** The average disease score in each group. Mice which died/were sacrificed during the experiment were given the same score for the rest of the experiment **C)** The average weight gain or loss for each group. Weights are compared with the weight on day 0. **D)** The mortality in each group. Only mice dead with EAE symptoms were included.

Figure *2* shows inhibition of EAE with compound A. Mice were immunized on day 0 with the $PLP_{139-153}$ peptide. Compound A were dosed as follows; compound A-50 (50 mg/kg); compound A-25 (25 mg/kg). Control groups were given either suspension vehicle i.p. from day 0 or dexamethasone (1 mg/kg) p.o. from day 1. The experiment was terminated on day 28 p.i. **A)** The frequency of animals with EAE symptoms. Mice dead or sacrificed during the experiment are included. **B)** The average disease score in each group. Mice which died/were

sacrificed during the experiment were given the same score for the rest of the experiment **C)** The average weight gain or loss for each group. The weights are compared with the weight on day 0. **D)** The mortality in each group. Only mice dead with EAE symptoms were included.

Figure 3 is a graph showing IL-2 production by spleen cells of mice immunized with $PLP_{139-153}$ peptide and dosed daily with compound A (n=6) or suspension vehicle (n=6). The spleen cells were collected on day 10 and restimulated *in vitro* with different concentrations of the $PLP_{139-153}$ peptide with or without compound A present (Fig. 3A and 3B, respectively). After 3 days of culture, the supernatants were tested for production of IL-2 using a time-resolved fluorometer. The average for each group is plotted together with the standard deviation.

Figure 4 is a graph showing cytokine production by spleen cells of mice immunized with $PLP_{139-153}$ peptide and dosed daily with compound A (n=4), compound D (n=4) or suspension vehicle (n=4). On day 10 spleen cells were collected and restimulated *in vitro* with different concentrations of the $PLP_{139-153}$ peptide. After 3 days culture the supernatants in each group were pooled and tested in duplicates for production of cytokines using a time-resolved fluorometer. The average stimulation index (SI) for each group is plotted together with the standard deviation. SI=(counts with peptide (0.5 mg/ml)/counts without peptide). Figure 4A shows the results for production of IL-2, figure 4B shows the results for production of IL-6, figure 4C shows the results for production of IFN-γ, and figure 4D shows the results for production of IL-17.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In the present context, the term "alkyl" is intended to indicate a univalent radical derived from straight or branched alkane by removing a hydrogen atom from any carbon atom. The alkane may include 1-10 carbon atoms, in particular 1-6 carbon atoms. The term includes the subclasses, primary, secondary and tertiary alkyl, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.-butyl, tert.-butyl, isopentyl and isohexyl.

The term "alkoxy" is intended to indicate a radical of formula OR*, wherein R* is alkyl as defined above, e.g. methoxy, ethoxy, propoxy, butoxy, etc.

The term "aryl" is intended to include radicals of carbocyclic aromatic rings, in particular 5- or 6-membered rings, optionally fused bicyclic rings, e.g. phenyl or naphthyl. The term

"heteroaryl" is intended to include radicals of heterocyclic aromatic rings, in particular 5- or 6-membered rings with 1-3 heteroatoms selected from O, S and N, or optionally fused bicyclic rings with 1-4 heteroatoms, e.g. pyridyl, triazolyl, tetrazolyl, thiazolyl, thiadiazolyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, oxatriazolyl, thienyl, pyrazinyl, isothiazolyl, indolyl, benzimidazolyl and benzofuranyl.

The term "alkylaryl" is intended to indicate an alkyl group covalently joined to an aryl group.

The term "sugar residue" is intended to indicate a glucuronide, e.g. hydroxyl or acyl glucuronide.

The term "halogen" is inteded to indicate fluoro, chloro, bromo or iodo.

The term "pharmaceutically acceptable salt" is intended to indicate salts prepared by reacting a compound of formula I with a suitable inorganic or organic acid, e.g. hydrochloric, hydrobromic, hydroiodic, sulfuric, nitric, acetic, phosphoric, lactic, maleic, phthalic, citric, propionic, benzoic, glutaric, gluconic, methanesulfonic, salicylic, succinic, tartaric, toluenesulfonic, sulfamic or fumaric acid.

The term "indolinone compound" is intended to include compounds of formula I, II, III or IV as shown above as well as other, structurally related compounds, such as the compounds disclosed in WO 96/40116, US 6,316,635, US 6,225,335, WO 99/48868, WO 99/61422, WO 01/60814, WO 00/56709, WO 01/83450, EP 934 931, US 5,834,504, WO 98/07695 and WO 02/02551 which are hereby incorporated by reference in their entirety. Methods of preparing the compounds are also disclosed in these publications.

The term "KDR" is understood to indicate a receptor tyrosine kinase which binds selectively to vascular endothelial growth factor (VEGF). The DNA and amino acid sequence of KDR as well as its proposed use to identify agonists and antagonists of VEGF action are disclosed in WO 92/14748.

The "src kinase family" is understood to indicate the tyrosine kinases src, lck, fyn, lyn, hck, fgr, blk and yes (J.B. Bolen and J.S. Brugge, *Ann. Rev. Immunol. 15*, 1997, p. 371).

The term "ameliorate" is intended to mean reducing the severity of the neurological symptoms during relapses of multiple sclerosis by administering an effective amount of an

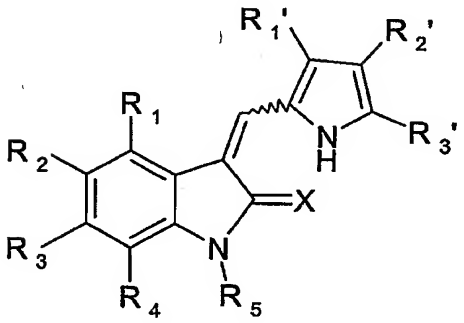
active compound whereby it may be possible to reduce or delay permanent disability resulting from neurological damage sustained during relapse, in particular demyelination.

The term "delay the onset of multiple sclerosis" is used to indicate a prophylactic administration of an effective amount of an active compound to prolong the period where no symptoms, or at least no severe symptoms, of multiple sclerosis are observed in susceptible individuals, e.g. in first-degree relatives of multiple sclerosis patients.

The term "reduce the relapse rate in multiple sclerosis" is intended to mean reducing the frequency with which relapses occur or, in other words, prolong the periods of remission. This may make it possible to reduce or delay the accumulation of disabilities resulting from the neurological damage sustained during each relapse, in particular demyelination which eventually leads to increasingly severe disability.

Preferred embodiments of the invention

In one embodiment, the invention relates to the use of a compound of general formula II



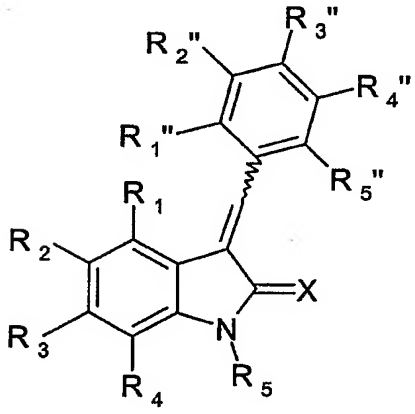
II

wherein R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;
R_5 is hydrogen or alkyl;
X is O or S; and
R_1', R_2' and R_3' are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH,

CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; and pharmaceutically acceptable salts thereof, for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay the onset of or reduce the relapse rate in multiple sclerosis.

Compounds of formula II are disclosed in WO 01/45689 in which they are indicated to be inhibitors of the *c-kit* protein tyrosine kinase and as such useful in the treatment of cell proliferative disorders such as cancer and mast cell proliferative disorders, e.g. mastocytosis, allergic rhinitis, inflammation and asthma. There is no suggestion that compounds within this group might have any utility in the treatment of multiple sclerosis.

In another embodiment, the invention relates to the use of compounds of general formula III



III

wherein R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

R_5 is hydrogen or alkyl;

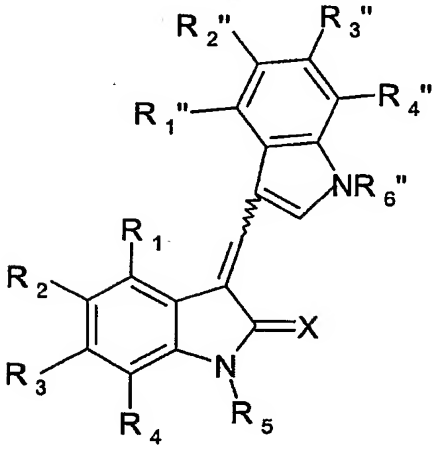
X is O or S; and

R_1", R_2", R_3", R_4" and R_5" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; and pharmaceutically acceptable salts thereof, for the preparation of a

medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay the onset of or reduce the relapse rate in multiple sclerosis.

Compounds of formula III are disclosed in WO 96/40116 in which they are indicated to be inhibitors of tyrosine kinases and as such useful in the treatment of cancer, blood vessel proliferative disorders, fibrotic disorders, mesangial cell proliferative disorders and metabolic diseases. There is no suggestion that compounds within this group might have any utility in the treatment of multiple sclerosis.

In a further embodiment, the invention relates to the use of compounds of formula IV



IV

wherein R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

R_5 is hydrogen or alkyl;

X is O or S;

R_1", R_2", R_3" and R_4" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; and

R_6" is hydrogen, alkyl, heteroaryl, heteroarylalkyl, C(O)R, COOR and SO_2R, wherein R is as indicated above; and pharmaceutically acceptable salts thereof, for the preparation of a

medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay the onset of or reduce the relapse rate in multiple sclerosis.

Compounds of formula IV are disclosed in WO 98/07695 in which they are indicated to be inhibitors of tyrosine kinases and as such useful in the treatment of cell proliferative diseases such as cancer, atherosclerosis, arthritis and restenosis, and metabolic diseases such as diabetes. There is no suggestion that compounds within this group might have any utility in the treatment of multiple sclerosis.

In the compound of formula I, R_5 is preferably hydrogen; X is preferably oxygen; R_1, R_2, R_3 and R_4 are preferably the same or different and independently selected from hydrogen and alkyl; and/or A is pyrrole, phenyl or indole, said pyrrole, phenyl or indole being optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above. In particular, A is pyrrole substituted at position 3 and 5 with alkyl.

In the compound of formula II, R_1, R_2, R_3 and R_4 are preferably the same or different and independently selected from hydrogen and alkyl, or R_2 is hydroxy or heteroaryl, such as pyridyl, or a group C(O)R, wherein R is heteroaryl, such as pyridyl or thienyl, and R_1, R_3 and R_4 are hydrogen; R_5 is preferably hydrogen; X is preferably O; and/or R_1', R_2' and R_3' are preferably the same or different and independently selected from hydrogen, alkyl, alkoxy, CH_2OH or COOH. In particular, R_1' and R_3' are both alkyl, in particular methyl, and R_2' is hydrogen, or R_1' is alkyl, in particular methyl, and R_3' is alkoxy, CH_2OH or COOH.

In the compound of formula III, R_1, R_2, R_3 and R_4 are preferably the same or different and independently selected from hydrogen and alkyl; R_5 is preferably hydrogen; X is preferably O; and/or R_1'', R_2'', R_3'', R_4'' and R_5'' are the same or different and independently selected from hydrogen, alkyl, alkoxy or halogen. In particular, R_2'' and R_5'' are independently alkyl, in particular methyl, or alkoxy, in particular methoxy.

In the compound of formula IV, R_1, R_2, R_3 and R_4 are preferably the same or different and independently selected from hydrogen and alkyl; R_5 is preferably hydrogen; X is preferably O; and/or R_1'', R_2'', R_3'' and R_4'' are the same or different and independently selected from hydrogen, alkyl, alkoxy or halogen.

Compounds of formula I are generally lipophilic in nature. While this property is believed to be advantage when in comes to passage across the blood-brain barrier, it makes the compounds sparingly soluble in water and consequently difficult to formulate in, for instance, parenteral, injectable compositions where isotonic saline is used as the solvent. To provide an adequate solubility of the compounds, they may advantageously be provided in the form of prodrugs. The term "prodrug" is intended to indicate a derivative of an active compound which does not, or does not necessarily, exhibit the physiological activity of the active compound, but which may be subjected to enzymatic cleavage such as hydrolysis *in vivo* so as to release the active compound on administration of the prodrug. In this particular instance, the prodrug comprises the active compound which in itself is highly lipophilic provided with a side chain with predominantly hydrophilic properties imparting improved solubility characteristics to the prodrug, thereby making it more suitable for parenteral administration in the form of a solution or for oral administration to obtain an improved bioavailability. Examples of prodrugs are compounds of formula I wherein R_5, apart from being hydrogen or alkyl, may also be a group $-C(R_6)NR_7R_8$, $-C(=O)NR_7R_8$, $-C(=O)R_9$, $-C(=O)OR_9$ or $-C(R_6)OR_{10}$, wherein R_6 is hydrogen or alkyl, R_7 and R_8 are the same or different and independently are hydrogen, alkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, phosphonooxyalkyl, sulfooxyalkyl, hydroxyalkyl, aryl, heteroaryl and heterocyclyl or, together with the N-atom to which they are attached, form a 5- or 6-membered ring, R_9 is alkyl, alkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, phosphonooxyalkyl, sulfooxyalkyl, hydroxyalkyl, aryl, heteroaryl, heteroarylalkyl, a sugar residue, heterocyclyl and heterocyclylalkyl, and R_{10} is hydrogen, alkyl, aralkyl, acyl or $-P(O)(OR)(OR')$. Such prodrugs and other, similar prodrugs may suitably be prepared by a procedure described in WO 01/90068, WO 01/90103, WO 01/90104 and WO 02/81466, the disclosures of which are hereby incorporated by reference in their entirety.

Currently favoured compounds of formula I are 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene-1,3-dihydro-indol-2-one, 3-(1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one and 3-(2,5-dimethoxy-benzylidene)-1,3-dihydroindol-2-one. These compounds have surprisingly been found to exhibit certain properties making them particularly favourable for the present purpose: they are highly lipophilic (logP 4.7) enabling them to cross the blood-brain barrier, and when tested on a number of different cytokines, they have been found to be potent inhibitors of the proinflammatory cytokines IL-2, IL-6, INF-γ and IL-17 (at a concentration of 10^{-10} M) while not inhibiting cytokines such as TNF-α. In case of the latter, it is interesting to note that a trial where anti-TNF antibodies were used in the treatment of multiple sclerosis actually led to an exacerbation of the symptoms (Karni and Weiner, *supra*). It would therefore appear to be an advantage to provide a treatment of multiple sclerosis which does not involve inhibition of TNF-α.

As indicated above, compounds of formula I have been described as tyrosine kinase inhibitors and, in particular, the compound 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one has been in development as an inhibitor of the vascular endothelial growth factor receptor KDR for the treatment of cancer. The compound has also been found to inhibit the p60c tyrosine kinase (L. Sun et al., *J. Med. Chem.* 43, 2000, p. 2655). In this context, it is interesting to note that compounds derived from a benzylidene malononitrile scaffold, termed tyrphostins, have been found to to be effective in the EAE model. Tyrphostins are known tyrosine kinase inhibitors. In particular, tyrphostin B42 (also termed AG490) has been found to inhibit IL-12 induced tyrosine phsophorylation and activation of JAK-2 kinase (cf. J.J. Bright et al., *J. Immunol.* 162, 1999, pp. 6255-6262). Furthermore, tyrphostin AG490 has been found to be an effective inhibitor of lymphocyte adhesion to inflamed vessels (cf. G. Constantin et al., *J. Immunol. 162*, 1999, pp. 1144-1149). It is suggested that the ability of AG 490 to block the development of EAE may be ascribed, at least in part, to its ability to inhibit lymphocyte adhesion.

It is recognised that tyrosine kinases play an important role in the regulation of cell signalling by phosphorylating tyrosine residues of proteins and peptides, and that excessive activation of tyrosine kinases may lead to the development of various diseases of the immune system. In this regards, members of the src kinase family have been found to be of interest, in particular the p56lck kinase which is only expressed in T-cells and which is crucial for T-cell receptor mediated signal transduction, eventually leading to production of proinflammatory cytokines, including IL-2. It has been found that T-cells which lack the p56lck kinase cannot signal through the T-cells receptor (D.B. Straus and A. Weiss, *Cell 70*, 1992, p. 585). As other tyrosine kinases are seemingly unable to compensate for blocking Lck activity (M.R. Myers et al., *Curr. Pharm. Design 3*, 1997, p. 473), this kinase may be an attractive target for therapeutic intervention.

Thus, in a still further embodiment, the invention relates to the use of a compound of general formula I capable of inhibiting a tyrosine kinase for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis or for the delay of the onset or relapse of multiple sclerosis. An example of such a compound is 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one which, when tested against a panel of tyrosine kinases (at Upstate Ltd., Dundee, UK), has been shown to inhibit not only the KDR tyrosine kinase as discussed above, but also the src kinase family members p56lck by about 50%, Fyn by about 30%, Yes by about 40%, Blk by about 40%, and Lyn by about 35%, at a concentration of 1 μM. As suggested above, members of the src kinase family are involved *inter alia* in T-cell receptor mediated signal transduction

pathways leading to activation of T-cells and are therefore believed to be attractive targets for medical intervention in immune and inflammatory diseases and conditions.

In this test, the compound 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one was also found to inhibit a number of other tyrosine kinases by at least 50%, including receptor tyrosine kinases such as the insulin receptor, PDGF receptor and FGF receptor; CDK-1, CDK-2 and CHK-2 which are involved in cell cycle; and MEK-1, RSK-1, RSK-2, RSK-3 and S6K involved in the mitogen-activated pathway.

As discussed above, proinflammatory cytokines produced by activated T-cells in the central nervous system are important factors in the demyelination process characteristic of multiple sclerosis. Proinflammatory cytokines are believed to participate directly in myelin destruction and axonal damage (O'Connor et al., *supra*) and also to play a role in the upregulation of MHC class II molecules on astrocytes and microglia as well as adhesion molecules on the blood-brain barrier endothelium, facilitating the further influx of T-cells, B-cells and macrophages in the central nervous system (Hellings et al., *supra*). Such cytokines may therefore be attractive targets for therapeutic intervention. In a further embodiment, the invention therefore relates to the use of a compound of general formula I capable of inhibiting the production of proinflammatory cytokines by T-cells or capable of blocking a cytokine receptor for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay the onset of or reduce the relapse rate in multiple sclerosis. Examples of such compounds are 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one and 3-(1H-indol-3-ylmethylene)-1,3-dihydro-indol-2-one) which, as discussed above, have been found to inhibit expression of IL-2, IL-6, INF-γ and IL-17 (cf. example 5 and figure 4A-4D).

When spleen cell cDNA derived from EAE mice treated with 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one and untreated EAE mice was tested in an oligonucleotide array of more than 5000 mouse genes (such as Atlas Plastic Mouse 5 K MicroArray, available from BD Biosciences, California, USA), a pattern emerged in cells from treated mice of downregulation of genes involved in the inflammatory response and upregulation of genes encoding adhesion molecules, suggesting that maturation and activation of inflammatory cells is retarted and consequently their migration into the CNS is reduced in the treated mice.

Pharmaceutical compositions

For use in the present invention, the active ingredient may be formulated into a pharmaceutical composition together with a pharmaceutically acceptable vehicle and optionally one or more other therapeutic ingredients. The vehicle must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The formulation may be in a form suitable for oral or parenteral (including subcutaneous, intramuscular, interperitoneal, intraarticular and intravenous) administration.

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy, e.g. as disclosed in Remington, The Science and Practise of Pharmacy, 20th Ed., 2000. All methods include the step of bringing the active ingredient into association with the vehicle which constitutes one or more auxiliary constituents. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient into association with a liquid vehicle or a finely divided solid vehicle or both, and then, if necessary, shaping the product into the desired formulation.

The term "dosage unit" is understood to mean a unitary, i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical vehicle materials.

Unlike IFN-β and glatiramer acetate which are peptidic in nature and only suitable for parenteral administration, such by injection, the compounds of formula I or II are small organic molecules and may therefore be administered orally. This represents a clear benefit for the patient as it permits self-medication and is less painful than for instance injections of IFN-β which often cause pain at the site of injection.

Formulations suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid, such as ethanol or glycerol; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. Such oils may be edible oils, such as e.g. cottonseed oil, sesame oil, coconut oil or peanut oil. Suitable dispersing or suspending agents for aqueous suspensions include synthetic or natural gums such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin,

methylcellulose and polyvinylpyrrolidone. The active ingredient may also be administered in the form of a bolus, electuary or paste.

A tablet may be prepared by compressing or moulding the active ingredient optionally with one or more auxiliary constituents. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient(s) in a free-flowing form such as a powder or granules, optionally mixed by a binder, such as e.g. lactose, glucose, starch, gelatine, acacia gum, tragacanth gum, sodium alginate, carboxymethylcellulose polyethylene glycol, waxes or the like; a lubricant such as e.g. sodium oleate, sodium stearate, magnesium steatrate, sodium benzoate, sodium acetate, sodium chloride or the like; a disintegrating agent such as e.g. starch, methyl cellulose, agar, bentonite, xanthan gum or the like or dispersing agent. Moulded tablets may be made by moulding, in a suitable machine, a mixture of the powdered active ingredient and suitable carrier moistened with an inert liquid diluent.

Formulations suitable for parenteral administration may conveniently comprise a sterile oily or aqueous preparation of the active ingredients, which is preferably isotonic with the blood of the recipient, e.g. an isotonic saline, isotonic glucose solution or buffer solution. Liposomal formulations may also be used to present the active ingredient for parenteral administration. The formulation may conveniently be sterilised by for instance filtration through a bacteria retaining filter, addition of sterilising agent to the formulation, irradiation of the formulation or heating of the formulation.

Alternatively, the formulation may be provided as a sterile, solid preparation, *e.g.* a freeze-dried powder, which is readily dissolved in a sterile media immediately prior to use.

In addition to the aforementioned ingredients, the formulations comprising a compound of formula I or II may include one or more additional ingredients such as diluents, buffers, flavouring agents, colourants, surface active agents, thickeners, preservatives, e.g. methyl hydroxybenzoate (including anti-oxidants), emulsifying agents and the like.

In addition to the formulations described above, compounds of formula I may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (*e.g.* subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the active ingredient may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in a pharmaceutically acceptable oil), or an ion exchange resin.

In a still further aspect, the present invention relates to a method of preventing, treating or ameliorating multiple sclerosis, or delaying the onset of or reducing the relapse rate in multiple sclerosis, the method comprising administering, to a patient in need thereof, a pharmacologically effective amount of a compound of general formula I. The invention further relates to a method of preventing, treating or ameliorating multiple sclerosis, or delaying the onset of or reducing the relapse rate in multiple sclerosis, the method comprising administering, to a patient in need thereof, a pharmacologically effective amount of a compound capable of inhibiting one or more tyrosine kinases or a compound capable of inhibiting the production of one or more proinflammatory cytokines by CD4+ Th1 cells, as discussed above.

For systemic treatment according to the present invention, daily doses of from 0.001-100 mg/kg body weight, preferably from 0.002-15 mg/kg body weight, for example 0.003-10 mg/kg of a compound of formula I or II are administered, typically corresponding to a daily dose for an adult human of from 0.2 to 750 mg of the active ingredient. Oral compositions are formulated, preferably as tablets, capsules, or drops, containing from 0.05-250 mg, preferably from 0.1-125 mg, of a compound of formula I per dosage unit.

EXAMPLES

Example 1

Materials and Methods

Compounds: Compound A = 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene-1,3-dihydro-indol-2-one; Compound B = 3-[2,4-dimethyl-5-(oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid; Compound C = a semisynthetic analogue of fumagillol, CAS RN 129298-91-5.

Peptide The following peptide from myelin proteolipid protein was used; $PLP_{139-153}$ H-HCLGKWLGHPDKFVG-OH. The peptide was synthesized by Fmoc chemistry (Schafer-N, Copenhagen, Denmark). Purity (>95%) was verified by reversed-phase HPLC and integrity by mass spectrometry.

Mice Female SJL/J (H-2^S) inbred mice purchased from Charles River.

Immunization The SJL/J mice (about 8 weeks old) were immunized on day 0 with the $PLP_{139-153}$ peptide (dissolved in sterile NaCl) emulsified 1:1 (vol/vol) in Complete Freund's Adjuvant (5 mg *Mycobacterium tuberculosis*/ml) (SSI, Copenhagen, Denmark). Intradermal injections corresponding to 100 µg peptide and 125 µg *Mycobacterium tuberculosis* were given at the base of the tail in a total volume of 50 µl. The mice were additionally given an i.v. injection with 100 ng pertussis toxin (Sigma) dissolved in sterile NaCl on day 0 and day 2, injection volume was 100 µl.

Compound treatment Groups of 10 mice were dosed daily with compound A (from 4 mg/kg to 50 mg/kg), compound B (from 50 mg/kg to 200 mg/kg) or compound C (5 mg/kg to 10 mg/kg) in suspension vehicle (4 g Tween-80, 2 g Carboxy-methyl cellulose 7H4XF, 8 g NaCl, 1 liter H_2O), starting on day 0 unless otherwise specified. Control groups were given either suspension vehicle or dexamethasone (Dexadreson Vet, Intervet, Holland). Suspension vehicle was given from day 0 i.p. whereas dexamethasone (0.5 or 1 mg/kg) was given p.o. from day 1.

Clinical evaluation Mice were weighed and assessed clinically daily from day 5 p.i. according to the following criteria: 0, no disease; 1, tail paralysis; 2, clumsy gait/poor righting ability and limb weakness; 3, moderate or total hind limb paralysis; 4, moribund state or dead.

Statistics

Area-under-curve (AUC) of the disease score was calculated for all mice. The medians of AUC of all groups were compared using Kruskal-Wallis test. When $P<0.05$ in the Kruskal-Wallis test, the Mann-Whitney test was used to compare drug treated groups with the suspension vehicle treated control group ($P<0.05$). AUC were calculated from day 0 to the termination of the individual experiment (day 21, day 28 or day 35).

Results

In four separate experiments, compound A (50 mg/kg) was shown to significantly inhibit EAE induced in SJL/J mice with a peptide from myelin proteolipid protein when compared with the suspension vehicle group (table 1, figure 1 and 2). Compound A is a known KDR inhibitor and has also been described to have an anti-angiogenic effect. In order to evaluate whether the inhibition of EAE could be ascribed to inhibition of KDR or inhibition of angiogenesis, a known KDR inhibitor and angiogenese inhibitor were also tested, compound B and compound C, respectively (table 1). There is no significant inhibition of EAE when mice are dosed with compound B or compound C indicating that a mechanism other than KDR and angiogenesis inhibition accounts for the effect of compound A. A dose-response

course is observed when compound A is given in different doses (table 1, figure 1) with a significant inhibition of EAE by dosing compound A as 50 mg/kg as wells as 25 mg/kg.

From the data obtained in experiment 3 (table 1, figure 2) and 4 (table 1) which were prolonged with one and two weeks repectively, it appears that treatment with compound A delays development of EAE rather than prevents the disease.

Table 1. Inhibition of EAE with compound A.

Exp.	Compound	AUC# (mean)	SD	inhibition (%)	Significance
1*	Vehicle	17.2	16.1		
	Dexamethasone from day 0 (0,5 mg/kg)	4.3	7,4	75	p=0.022
	A (50 mg/kg i.p.)	1.8	5.5	90	p<0.01
	B (50 mg/kg i.p.)	23.4	12.5	-36	NS
2**	Vehicle	25.4	9,0		
	Dexamethasone from day 1 (1 mg/kg)	11.8	8.1	54	p<0.01
	A (50 mg/kg i.p.)	0.6	1.7	98	p<0.01
	A (25 mg/kg i.p.)	4.6	7.7	82	p<0.01
	A (10 mg/kg i.p.)	19.6	8.7	23	NS
	A (4 mg/kg i.p.)	21.2	14.5	17	NS
	C (10 mg/kg i.p.)	19.3	12.7	24	NS
	C (5 mg/kg i.p.)	19.0	13.6	25	NS
3***	Vehicle	39.7	17.9		
	Dexamethasone from day 1 (1 mg/kg)	0.8	2.2	98	p<0.01
	A (50 mg/kg i.p.)	6.6	7.8	83	p<0.01
	A (25 mg/kg i.p.)	11.9	7.8	70	p<0.01
4****	Vehicle	67.2	37.0		
	A (50 mg/kg i.p.)	21.9	19.7	65	p=0.011
	B (200 mg/kg p.o.)	55.5	36.5	11	NS

Area-under-curve of the disease score for four individually experiments. In each experiment a suspension vehicle group was included. AUC of the disease score were calculated for all groups and the Mann-Whitney test was used to compare the drug treated groups with the suspension vehicle group.

*This experiment was terminated on day 21. **This experiment was terminated on day 21. ***This experiment was terminated on day 28. ****This experiment was terminated on day 35. #AUC for the experiments are calculated from day 0 to termination of the individual experiment.

Example 2

Inhibition of IL-2 production in spleen cells of mice dosed with Compound A

Materials and methods

Peptide The following peptide from myelin proteolipid protein was used; $PLP_{139-153}$ H-HCLGKWLGHPDKFVG-OH. The peptide was synthesized by Fmoc chemistry (Schafer-N, Copenhagen, Denmark). Purity (>95%) was verified by reversed-phase HPLC and integrity by mass spectrometry.

Mice Female SJL/J ($H\text{-}2^S$) inbred mice purchased from Charles River.

Immunization The SJL/J mice (about 8 weeks old) were immunized on day 0 with the $PLP_{139-153}$ peptide (dissolved in sterile NaCl) emulsified 1:1 (vol/vol) in Complete Freund's Adjuvant (5 mg *Mycobacterium tuberculosis*/ml) (SSI, Copenhagen, Denmark). Intradermal injections corresponding to 100 µg peptide and 125 µg *Mycobacterium tuberculosis* were given at the base of the tail in a total volume of 50 µl. The mice were additionally given an i.v. injection with 100 ng pertussis toxin (Sigma) dissolved in sterile NaCl on day 0 and day 2, injection volume was 100 µl.

Compound treatment A group of 6 mice were dosed i.p. daily for 10 days with compound A (50 mg/kg), in suspension vehicle (4 g Tween®-80, 2 g carboxymethylcellulose 7H4XF, 8 g NaCl, 1 liter H_2O), starting on day 0. A control group was given suspension vehicle daily.

Clinical evaluation Mice were weighed and assessed clinically daily from day 5 p.i. according to the following criteria: 0, no disease; 1, tail paralysis; 2, clumsy gait/poor righting ability and limb weakness; 3, moderate or total hind limb paralysis; 4, moribund state or dead.

In vitro restimulation Ten days after immunization individual spleens were collected and single-cell suspensions were prepared in RPMI (Bio Whittaker) supplemented with 1% mouse serum, 1% penicillin/streptomycin (Gibco) and 1 % L-glutamine (Gibco). The erythrocytes were lysed by 0.83% NH_4Cl treatment. Subsequently the cells were washed and plated out ($5X10^5$ cells/well) on 96-well flat-bottomed microtiter plates (Nunclon, NUNC, Denmark). Different concentrations of the $PLP_{139-153}$ peptide were added to the wells. Control wells did not contain the $PLP_{139-153}$ peptide. Cells from each mouse were plated with or without 10^{-7}M of compound A on the microtiter plate. The plates were incubated at 37 °C

for 3 days. Afterwards 100 µl of the supernatant from each well was transferred to a corresponding 96-well flat-bottomed microtiter plate (Maxisorp, NUNC, Denmark) coated with 50 µl/well of an anti-IL-2 antibody (2 µg/ml) (PharMingen, Becton Dickinson) in coating buffer (sodium bicarbonate, 0.1 mol/L, pH 9.5). The Maxisorp plates were blocked with blocking buffer (10% FCS in PBS) and washed with (0.05 % Tween®-20 in PBS) before addition of the supernatant. After incubation overnight at 4 °C the plates were washed and 100 µl of biotinylated anti-IL-2 antibody (1 µg/ml) (PharMingen, Becton Dickinson), diluted in blocking buffer, was added to each well. After 45 min. incubation at room temperature, the plates were washed and incubated for 2 hrs. at room temperature on a plate-shaker with 100 µl/well of EU^{3+}-labeled streptavidin (Wallac, Perkin Elmer) diluted 1/1000 in assay buffer (Wallac, Perkin Elmer). After washing, the plates were incubated for 10 min. on a plate-shaker with 180 µl enhancement solution (Wallac, Perkin Elmer) which releases europium from streptavidin and forms a highly fluorescent micellar solution. Finally, fluorescence was measured in a time-resolved fluorometer VICTOR (Wallac, Perkin Elmer).

Conclusion

Two separate *in vitro* restimulation experiments (one example is given) show that the IL-2 production is markedly inhibited *in vivo* after dosing with compound A (50 mg/kg) for 10 days (Fig. 3A). The inhibition of IL-2 is antigen dependent as there were no IL-2 production in wells without $PLP_{139-153}$ (data not shown). Normal mice (not immunized) were also included in the experiment and did not produce any IL-2 after incubation with or without $PLP_{139-153}$ (data not shown). Furthermore, the results show that incubation for 3 days *in vitro* with 10^{-7} M of compound A did not inhibit the IL-2 production from cells originating from the suspension vehicle treated mice (Fig. 3B).

Example 3

Inhibition of EAE in a second model for EAE

Materials and Methods

Compounds: Compound A = 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one

Peptid The following peptide from myelin oligodendrocyte glycoprotein was used; MOG_{35-55} H-MEVGWYRSPFSRVVHLYRNGK-OH. The peptide was synthesized by Fmoc chemistry

(Schafer-N, Copenhagen, Denmark). Purity (>95%) was verified by reversed-phase HPLC and integrity by mass spectrometry.

Mice Female C57BL/6JBom (H-2^b) inbred mice purchased from Taconic M&B, Denmark.

Immunization The C57BL/6JBom mice (about 8 weeks old) were immunized at day 0 with the MOG_{35-55} peptide (dissolved in sterile NaCL) emulsified 1:1 (vol/vol) in Complete Freund's Adjuvant (5 mg *Mycobacterium tuberculosis*/ml) (Statens Serum Institut, Copenhagen, Denmark). Intradermal injections corresponding to 200 μg peptide and 250 μg *Mycobacterium tuberculosis* were given at the base of the tail in a total volume of 50 μl. The mice were additionally given an i.v. injection with 100 ng Pertussis Toxin (Sigma) dissolved in sterile NaCl at day 0 and day 2, injection volume was 100 μl.

Compound treatment Groups of 10 mice were dosed daily with compound A (50 mg/kg i.p.) in suspension vehicle (4 g Tween-80, 2 g carboxy-methyl cellulose 7H4XF, 8 g NaCl, 1 liter H_2O) starting at day 0. Control groups were given either suspension vehicle or dexamethasone (Dexadreson Vet, Intervet, Holland). Suspension vehicle was given from day 0 i.p. whereas dexamethasone (1 mg/kg) was given p.o. from day 1.

Clinical evaluation Mice were weighed and assessed clinically daily from day 5 p.i. according to the following criteria: 0, no disease; 1, tail paralysis; 2, clumsy gait/poor righting ability and limb weakness; 3, moderate or total hind limb paralysis; 4, moribund state or dead.

Statistics

Area under curve (AUC) of the disease score was calculated for all mice. The medians of AUC of all groups were compared using Kruskal-Wallis test. When $p<0.05$ in the Kruskal-Wallis test, the Mann-Whitney test was used to compare drug treated groups with the suspension vehicle treated control group ($p<0.05$). AUC were calculated from day 0 to the termination of the experiment (day 21).

Results

The results presented in table 2 demonstrate that compound A has a significant effect on disease development in C57BL/6 mice immunized with a peptide from myelin oligodendrocyte glycoprotein, indicating that the effect of compound A on EAE is

independent of the mouse strain used in the experiment and the immunization protocol used for the induction of EAE.

Table 2 Inhibition of EAE with compound A in a second EAE model

Compound	AUC# (mean)	SD	Inhibition (%)	Significance
Vehicle	15.40	8.4		
Dexamethasone (1 mg/kg p.o.)	0.00	0.0	100	p<0.01
Compound A (50 mg/kg i.p.)	2.65	6.1	83	p<0.01

Area-under-curve of the disease score. AUC of the disease score were calculated for all groups and the Mann-Whitney test was used to compare the treated groups with the suspension vehicle group.

Example 4

Inhibition of EAE with analogs of compound A

Materials and Methods

Compounds: Compound D= 3-(1H-indol-3-ylmethylene)-1,3-dihydro-indol-2-one) and Compound E= 3-(2,5-dimethoxybenzylidene)-1,3-dihydro-indol-2-one.

Peptide The following peptide from myelin proteolipid protein was used; $PLP_{139-153}$ H-HCLGKWLGHPDKFVG-OH. The peptide was synthesized by Fmoc chemistry (Schafer-N, Copenhagen, Denmark). Purity (>95%) was verified by reversed-phase HPLC and integrity by mass spectrometry.

Mice Female SJL/J ($H\text{-}2^S$) inbred mice purchased from Charles River or Taconic M&B (Denmark).

Immunization The SJL/J mice (about 8 weeks old) were immunized at day 0 with the $PLP_{139-153}$ peptide (dissolved in sterile NaCL) emulsified 1:1 (vol/vol) in Complete Freund's Adjuvant (5 mg *Mycobacterium tuberculosis*/ml) (Statens Serum Institut, Copenhagen, Denmark). Intradermal injections corresponding to 100 μg peptide and 125 μg *Mycobacterium tuberculosis* were given at the base of the tail in a total volume of 50 μl. The

mice were additionally given an i.v. injection with 100 ng Pertussis Toxin (Sigma) dissolved in sterile NaCl at day 0 and day 2, injection volume was 100 µl.

Compound treatment Groups of 10 mice were dosed daily with compound D (50 mg/kg) or compound E (50 mg/kg) in suspension vehicle (4 g Tween-80, 2 g Carboxy-methyl cellulose 7H4XF, 8 g NaCl, 1 liter H_2O), starting at day 0. Control groups were given either suspension vehicle or dexamethasone (Dexadreson Vet, Intervet, Holland). Suspension vehicle was given from day 0 i.p. whereas dexamethasone (1 mg/kg) was given p.o. from day 1.

Clinical evaluation Mice were weighed and assessed clinically daily from day 5 p.i. according to the following criteria: 0, no disease; 1, tail paralysis; 2, clumsy gait/poor righting ability and limb weakness; 3, moderate or total hind limb paralysis; 4, moribund state or dead.

Statistics

Area under curve (AUC) of the disease score was calculated for all mice. The medians of AUC of all groups were compared using Kruskal-Wallis test. When $p<0.05$ in the Kruskal-Wallis test, the Mann-Whitney test was used to compare drug treated groups with the suspension vehicle treated control group ($p<0.05$). AUC were calculated from day 0 to the termination of the experiment (day 21).

Results

Analogs of the potent compound A were tested in the EAE model in order to further evaluate the *in vivo* effect of this group of compounds. The results are shown in table 3 from which it appears that the two analogs compound D and compound E both show a clear and significant inhibition of the disease.

Table 3 Inhibition of EAE with compounds D and E

Compound	AUC# (mean)	SD	Inhibition (%)	Significance
Vehicle	28.6	9.5		
Dexamethasone (1 mg/kg p.o.)	13.15	10.7	54	p<0.01
Compound D (50 mg/kg i.p.)	4.70	6.5	84	p<0.01

Vehicle	42.75	5.3		
Dexamethasone (1 mg/kg p.o.)	8.85	8.6	79	p<0.01
Compound E (50 mg/kg i.p.)	12.15	11.8	72	p<0.01

Area-under-curve of the disease score. A suspension vehicle group was included. AUC of the disease score were calculated for all groups and the Mann-Withney test was used to compare the treated groups with the suspension vehicle group.

Example 5

Inhibition of pro-inflammatory cytokines

Materials and Methods

Peptide The following peptide from myelin proteolipid protein was used; $PLP_{139-153}$ H-HCLGKWLGHPDKFVG-OH. The peptide was synthesized by Fmoc chemistry (Schafer-N, Copenhagen, Denmark). Purity (>95%) was verified by reversed-phase HPLC and integrity by mass spectrometry.

Mice Female SJL/J ($H\text{-}2^S$) inbred mice purchased from Charles River.

Immunization The SJL/J mice (about 8 weeks old) were immunized at day 0 with the $PLP_{139-153}$ peptide (dissolved in sterile NaCl) emulsified 1:1 (vol/vol) in Complete Freund's Adjuvant (5 mg *Mycobacterium tuberculosis*/ml) (Statens Serum Institut, Copenhagen, Denmark). Intradermal injections corresponding to 100 μg peptide and 125 μg *Mycobacterium tuberculosis* were given at the base of the tail in a total volume of 50 μl. The mice were additionally given an i.v. injection with 100 ng Pertussis Toxin (Sigma) dissolved in sterile NaCl at day 0 and day 2, injection volume was 100 μl.

Compound treatment Groups of 4 mice were dosed i.p. daily for 10 days with compound A (50 mg/kg) or compound D (50 mg/kg) in suspension vehicle (4 g Tween-80, 2 g Carboxy-methyl cellulose 7H4XF, 8 g NaCl, 1 liter H_2O), starting at day 0. A control group were given suspension vehicle daily.

Clinical evaluation Mice were weighed and assessed clinically daily from day 5 p.i. according to the following criteria: 0, no disease; 1, tail paralysis; 2, clumsy gait/poor righting ability and limb weakness; 3, moderate or total hind limb paralysis; 4, moribund state or dead.

In vitro restimulation Ten days after immunization individual spleens were collected and single-cell suspensions were prepared in RPMI (Bio Whittaker) supplemented with 1% mouse serum, 1% penicillin/streptomycin (Gibco) and 1 % L-glutamine (Gibco). The erythrocytes were lysed by 0.83% NH_4CL treatment. Subsequently the cells were washed and plated out ($5X10^5$ cells/well) on 96-well flat-bottomed microtiter plates (Nunclon, NUNC, Denmark). Different concentrations of the $PLP_{139-153}$ peptide were added to the wells. Control wells were without the $PLP_{139-153}$ peptide. The plates were incubated at 37 °C for 3 days. Afterwards the supernatants from each mouse were pooled within the respective group and frozen for later analysis. For analysis the supernatants were transferred to a 96-well flat-bottomed microtiter plate (Maxisorp, NUNC, Denmark) coated with 50 µl/well of an anti-cytokine antibody (table 4) in coating buffer (natriumbicarbonate, 0.1 mol/L, pH 9.5). The Maxisorp plates were blocked with blocking buffer (10% FCS in PBS) and washed with (0.05 % Tween-20 in PBS) before addition of the supernatant. After incubation O/N at 4 °C the plates were washed and 100 µl of biotinylated anti-cytokine antibody (table 4) diluted in blocking buffer was added to each well. After 45 min. incubation at room temperature, the plates were washed and incubated for 2 hr. at room temperature on a plate-shaker with 100 µl/well of EU^{3+}-labeled streptavidin (Wallac, Perkin Elmer) diluted 1/1000 in assay buffer (Wallac, Perkin Elmer). After washing, the plates were incubated for 10 min. on a plate-shaker with 180 µl enhancement solution (Wallac, Perkin Elmer) which releases europium from streptavidin and forms a highly fluorescent micellar solution. Finally, fluorescence was measured in a time-resolved fluorometer VICTOR (Wallac, Perkin Elmer).

Table 4: dilution of used anti-cytokine antibodies

Cytokine	Company	Format	Working concentration
IL-2	BD Biosciences	Purified Ab	2 µg/ml
		Biotinylated Ab	1 µg/ml
IL-6	BD Biosciences	Purified Ab	2 µg/ml
		Biotinylated Ab	1 µg/ml
IL-17	BD Biosciences	Purified Ab	10 µg/ml
		Biotinylated Ab	5 µg/ml
IFN-γ	BD Biosciences	Purified Ab	10 µg/ml
		Biotinylated Ab	5 µg/ml

Results

It appears from Fig. 4A-4D that there is a pronounced difference in the level of antigen-specific cytokine production of spleen cells *in vivo* after treatment of immunized mice with either compound A or compound D (compound E not tested) compared to the vehicle treated group. The level of the cytokines IL-2, IL-6, IFN-γ and IL-17 are clearly reduced when mice have been dosed with either of these two compounds which also inhibits the disease cause very efficiently. The reduction in one or more of these pro-inflammatory cytokines can be involved in the disease inhibiting process.

Example 6

Differential up- and downregulation of genes in EAE mice treated with compound A compared to untreated controls

RNA extraction, probe synthesis and hybridization

SJL mice immunized with an encephalitogenic epitope of the proteolipid protein ($PLP_{139-153}$) were dosed daily with compound A from day 0 as described in example 1. Control mice were given suspension vehicle as described in example 1. On day 7 after treatment, the mice (both treated and control) were sacrificed and their spleens were excised, submerged into RNA-later (Sigma-Aldrich, St. Louis, USA) and stored at -80°C. Total RNA was extracted from the mouse spleens using the TRIZOL extraction procedure according to the manufacturer's protocol followed by DNAse treatment (Invitrogen, Carlsbad, CA, USA). The RNA quality was visualized by agarose gel electrophoresis. Ten µg total RNA was converted into purified ^{33}P-labelled cDNA probe according to the Atlas Pure Total RNA labelling protocol (BD Biosciences, Palo Alta, CA, USA).

Labelled probes (10×10^6 CPM) were denatured prior to hybridization to oligonucleotide arrays comprising over 5.000 mouse genes (Atlas Plastic Mouse 5K MicroArray, BD Biosciences, Palo Alta, CA, USA). Arrays were hybridized for 16h at 60°C with rotation. After hybridization and appropriate washing steps, the arrays were imaged using phospho imager technology together with a STORM 860 scanner (Molecular Dynamics, Sunnyvale, CA, USA). Subsequently, the images were analysed using the Atlas Image 2.7 software (BD Biosciences, Palo Alta, CA, USA).

Results

Approximately 2% of the genes located on the Atlas MicroArray were differentially expressed when arrays from the spleen cDNA of treated and control mice were compared. Several immune response genes were found to be upregulated (class II major histocompatilibility complex [NM_010379, NM_010388]), immunoglobulin-associated beta [NM_008339], transforming growth factor beta-3 [NM_009368], Fc receptor (IgE, high affinity I, gamma polypeptide [NM_010185]) and beta-2 microglubulin [NM_009735]) in the treated group. Genes encoding adhesion molecules (lymphocyte antigens 6 locus D [NM_010742], lymphocyte antigens 6 locus E [NM_008529] and lymphocyte antigens 84 [NM_010743]) were found to be upregulated in the treated group. Genes encoding the interleukin 1 receptor [M20658] and the interleukin 10 receptor alpha [L12120] together with genes encoding tumor necrosis factor related proteins (TNF receptor [M59378] and TNF receptor-associated factor 1 [NM_009421]) were downregulated in the treated group. Upregulation of genes encoding adhesion molecules as well as downregulation of genes involved in the inflammatory response (interleukin and TNF related genes) suggests that maturation and activation of inflammatory cells is reduced as is their migration into the CNS.

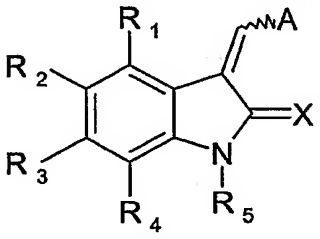
Genes related to anti-apoptosis (Bcl-2 like protein [U51279] and elastase [NM_015779]) were found to be downregulated in the treated group allowing apoptosis of inflammatory cells to take place.

Genes having a proposed role in re-myelination were found to be differentially expressed (complement factor [NM_009777], myelin transcription factor [AF004294], fibroblast growth factor 15 [AF007268]) in treated and control mice, indicating a role of compound A in the regression of symptoms of EAE and consequent remyelination of axons.

The figures in square brackets above refer to the GenBank Accession No. of the respective genes.

CLAIMS

1. Use of a compound of general formula I



I

wherein

X is O or S;

R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

A is phenyl or a monoclyclic or bicyclic heteroaryl ring selected from the group consisting of pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, tetrazole and indole, optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above, the zigzag line indicating that the group denoted A is present as the E- or Z-isomer;

R_5 is hydrogen or alkyl;

or pharmaceutically acceptable salts thereof, for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay of the onset of or reduce the relapse rate in multiple sclerosis.

2. The use of claim 1 wherein, in the compound of formula I, R_5 is hydrogen.

3. The use of claim 1 wherein, in the compound of formula I, X is oxygen.

4. The use of claim 1 wherein, in the compound of formula I, R_1, R_2, R_3 and R_4 are the same or different and independently selected from hydrogen and alkyl.

5. The use of any of claims 1-4 wherein, in the compound of formula I, A is pyrrole, phenyl or indole, said pyrrole, phenyl or indole being optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated in claim 1.

6. The use of claim 5 wherein, in the compound of formula I, A is pyrrole substituted at position 3 and 5 with alkyl, or at position 3 with alkyl and at position 5 with CH_2OH, COOH or a sugar residue.

7. The use of claim 5 wherein, in the compound of formula I, A is phenyl substituted at position 2 and 5 with alkyl, alkoxy or halogen.

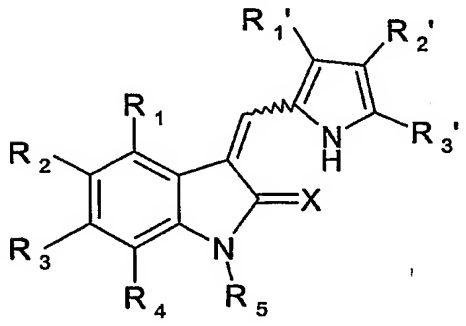
8. The use of claim 5 wherein, in the compound of formula I, A is indole.

9. The use of claim 6 wherein the compound is 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene-1,3-dihydro-indol-2-one.

10. The use of claim 7 wherein the compound is 3-(2,5-dimethoxy-benzylidene)-1,3-dihydroindol-2-one.

11. The use of claim 8 wherein the compound is 3-(1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one.

12. The use of claim 1, wherein the compound is a compound of general formula II



II

wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 1, and R_1', R_2' and R_3' are R_1', R_2' and R_3' are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; or pharmaceutically acceptable salts thereof.

13. The use of claim 12 wherein, in the compound of formula II, R_1, R_2, R_3 and R_4 are the same or different and independently selected from hydrogen and alkyl, or R_2 is hydroxy or heteroaryl, such as pyridyl, or a group C(O)R, wherein R is heteroaryl, such as pyridyl or thienyl, and R_1, R_3 and R_4 are hydrogen.

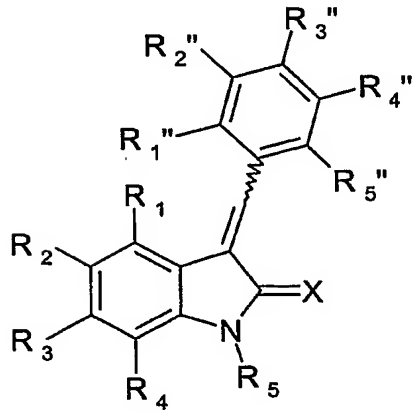
14. The use of claim 12 wherein, in the compound of formula II, R_1', R_2' and R_3' are the same or different and independently selected from hydrogen, alkyl, alkoxy, CH_2OH or COOH.

15. The use of claim 14 wherein R_1' and R_3' are both alkyl, in particular methyl, and R_2' is hydrogen, or wherein R_1' is alkyl and R_3' is alkoxy, CH_2OH or COOH.

16. The use of claim 15 wherein the compound is 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one.

17. The use of claim 1, wherein the compound is a compound of formula III

III



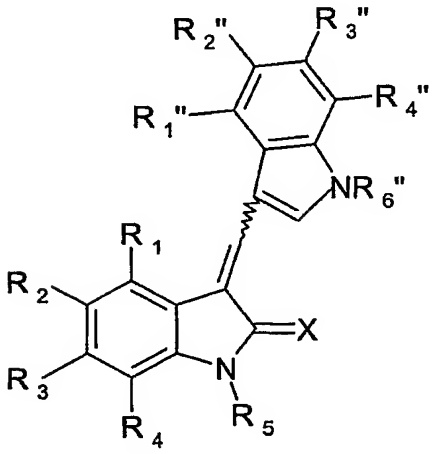
III

wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 1, and R_1", R_2", R_3", R_4" and R_5" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; or pharmaceutically acceptable salts thereof.

18. The use of claim 17 wherein R_2" and R_5" are the same or different and independently are alkyl, in particular methyl, or alkoxy, in particular methoxy.

19. The use of claim 18, wherein the compound is 3-(2,5-dimethoxy-benzylidene)-1,3-dihydroindol-2-one.

20. The use of claim 1 wherein the compound is a compound of general formula IV

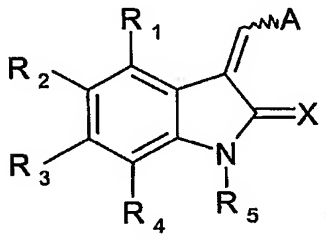


IV

wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 1, R_1", R_2", R_3" and R_4" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; and R_6" is hydrogen, alkyl, heteroaryl, heteroarylalkyl, C(O)R, COOR or SO_2R; or pharmaceutically acceptable salts thereof.

21. The use of claim 20 wherein the compound is 3-(1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one.

22. A method of preventing, treating or ameliorating multiple sclerosis, or delaying the onset of or reducing the relapse rate in multiple sclerosis, the method comprising administering, to a patient in need thereof, a pharmacologically effective amount of a compound of general formula I



I

wherein

X is O or S;

R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

A is phenyl or a monoclyclic or bicyclic heteroaryl ring selected from the group consisting of pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, tetrazole and indole, optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above, the zigzag line indicating that the group denoted A is present as the E- or Z-isomer;

R_5 is hydrogen or alkyl;

or pharmaceutically acceptable salts thereof.

23. The method of claim 22 wherein, in the compound of formula I, R_5 is hydrogen.

24. The method of claim 22 wherein, in the compound of formula I, X is oxygen.

25. The method of claim 22 wherein, in the compound of formula I, R_1, R_2, R_3 and R_4 are the same or different and independently selected from hydrogen and alkyl.

26. The method of claim 22 wherein, in the compound of formula I, A is pyrrole, phenyl or indole, said pyrrole, phenyl or indole being optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated in claim 22.

27. The method of claim 26 wherein, in the compound of formula I, A is pyrrole substituted at position 3 and 5 with alkyl, or at position 3 with alkyl and at position 5 with CH_2OH, COOH or a sugar residue.

28. The method of claim 26 wherein, in the compound of formula I, A is phenyl substituted at position 2 and 5 with alkyl, alkoxy or halogen.

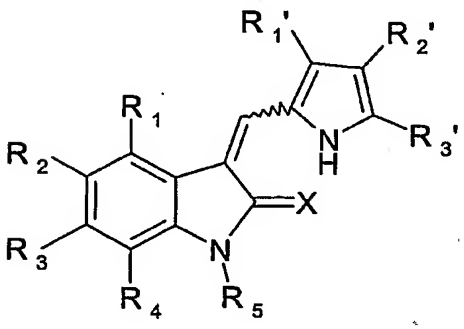
29. The method of claim 26 wherein, in the compound of formula I, A is indole.

30. The the method of claim 27 wherein the compound is 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene)-1,3-dihydro-indol-2-one.

31. The method of claim 28 wherein the compound is 3-(2,5-dimethoxy-benzylidene)-1,3-dihydroindol-2-one.

32. The method of claim 29 wherein the compound is 3-(1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one.

33. The method of claim 22 wherein the compound is a compound of general formula II



II

wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 22, and R_1', R_2' and R_3' are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; or pharmaceutically acceptable salts thereof.

34. The method of claim 33 wherein, in the compound of formula II, R_1, R_2, R_3 and R_4 are the same or different and independently selected from hydrogen and alkyl, or R_2 is heteroaryl, such as pyridyl, or a group C(O)R, wherein R is heteroaryl, such as pyridyl or thienyl, and R_1, R_3 and R_4 are hydrogen.

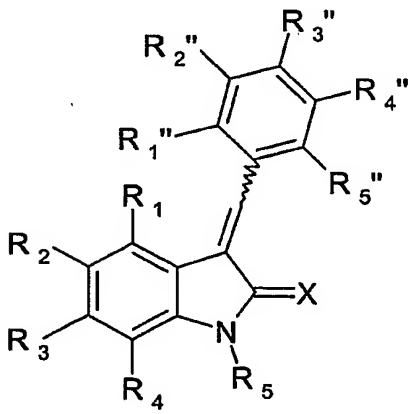
35. The method of claim 33 wherein, in the compound of formula II, R_1', R_2' and R_3' are the same or different and independently selected from hydrogen, alkyl, alkoxy, CH_2OH or COOH.

36. The method of claim 35 wherein R_1' and R_3' are both alkyl, in particular methyl, and R_2' is hydrogen, or wherein R_1' is alkyl and R_3' is alkoxy, CH_2OH or COOH.

37. The method of claim 36 wherein the compound is 3-(3,5-dimethyl-1H-pyrrol-2-yl-methylene-1,3-dihydro-indol-2-one.

38. The method of claim 22 wherein the compound is a compound of formula III

III



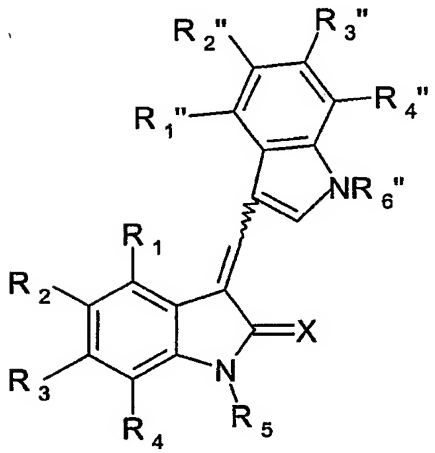
III

wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 22, and R_1", R_2", R_3", R_4" and R_5" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; or pharmaceutically acceptable salts thereof.

39. The method of claim 38 wherein R_2" and R_5" are the same or different and independently are alkyl, in particular methyl, or alkoxy, in particular methoxy.

40. The method of claim 39 wherein the compound is 3-(2,5-dimethoxy-benzylidene)-1,3-dihydroindol-2-one.

41. The method of claim 22 wherein the compound is a compound of general formula IV



IV

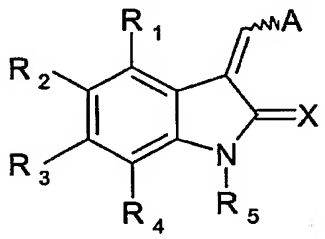
wherein R_1, R_2, R_3, R_4, R_5 and X are as indicated in claim 22, and R_1", R_2", R_3" and R_4" are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, OC(O)R, COOR, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above; and R_6" is hydrogen, alkyl, heteroaryl, heteroarylalkyl, C(O)R, COOR or SO_2R; or pharmaceutically acceptable salts thereof.

42. The method of claim 41 wherein the compound is 3-(1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one.

N vel Therapeutic Use

ABSTRACT

Compounds of general formula I



I

wherein

X is O or S;

R_1, R_2, R_3 and R_4 are the same or different and independently selected from the group consisting of hydrogen, alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R is hydrogen, alkyl, heteroaryl or aryl, R' is hydrogen, alkyl or aryl, and n is 0-3;

A is phenyl or a monoclyclic or bicyclic heteroaryl ring selected from the group consisting of pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, tetrazole and indole, optionally substituted at one or more positions with alkyl, alkoxy, aryl, heteroaryl, aryloxy, alkylaryl, alkylaryloxy, halogen, trihalomethyl, a sugar residue, S(O)R, SO_2R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, CH_2OH, C(O)R, COOR, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$ and CONRR', wherein R, R' and n are as indicated above, the zigzag line indicating that the group denoted A is present as the E- or Z-isomer;

R_5 is hydrogen or alkyl;

or pharmaceutically acceptable salts thereof, for the preparation of a medicament for the prevention, treatment or amelioration of multiple sclerosis, or to delay of the onset of or reduce the relapse rate in multiple sclerosis.

Figure 1

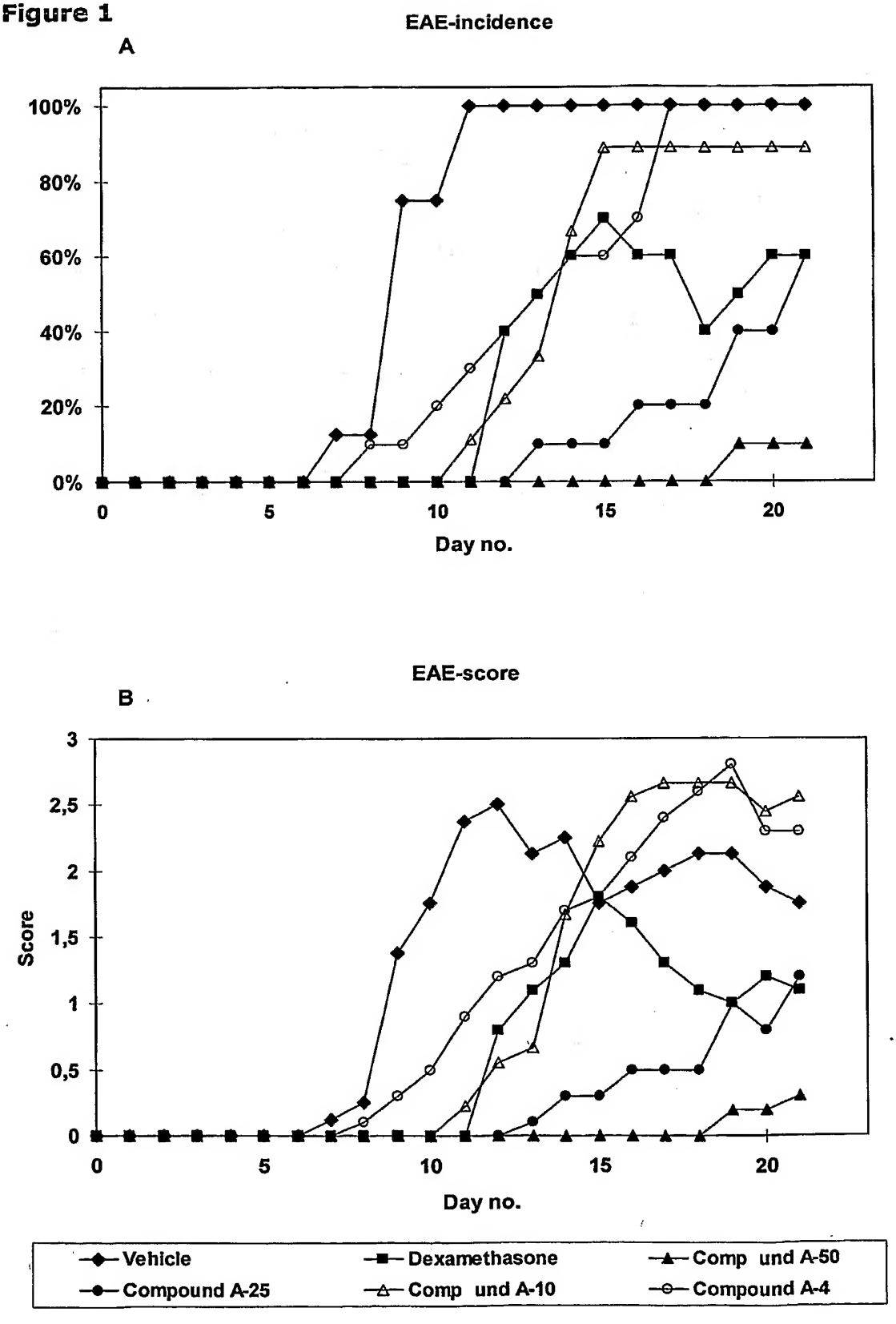
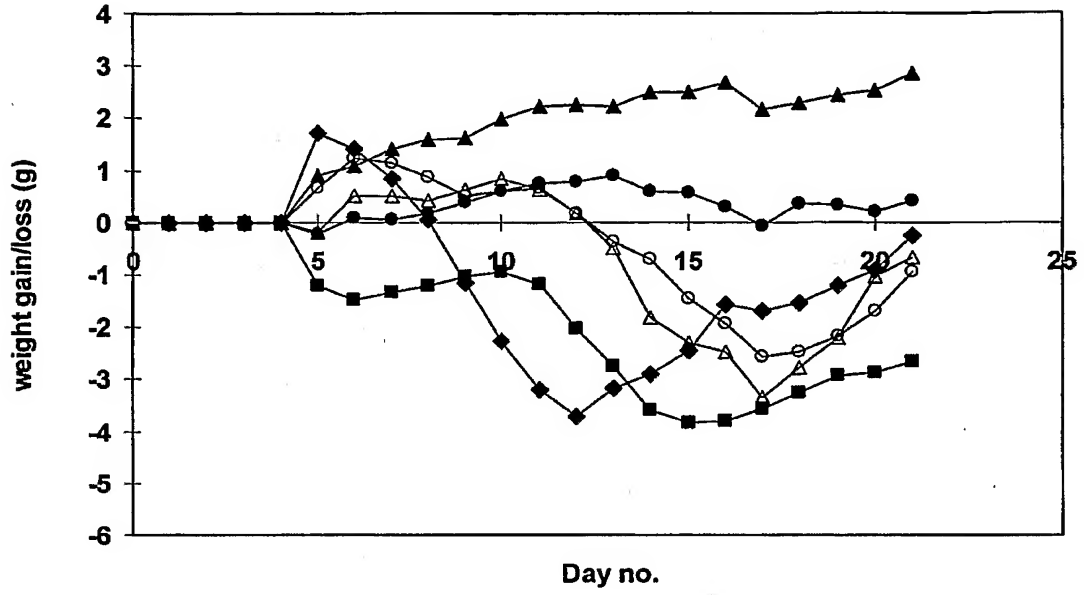


Figure 1 EAE-weight gain/loss compared to day 0

C



D EAE-mortality

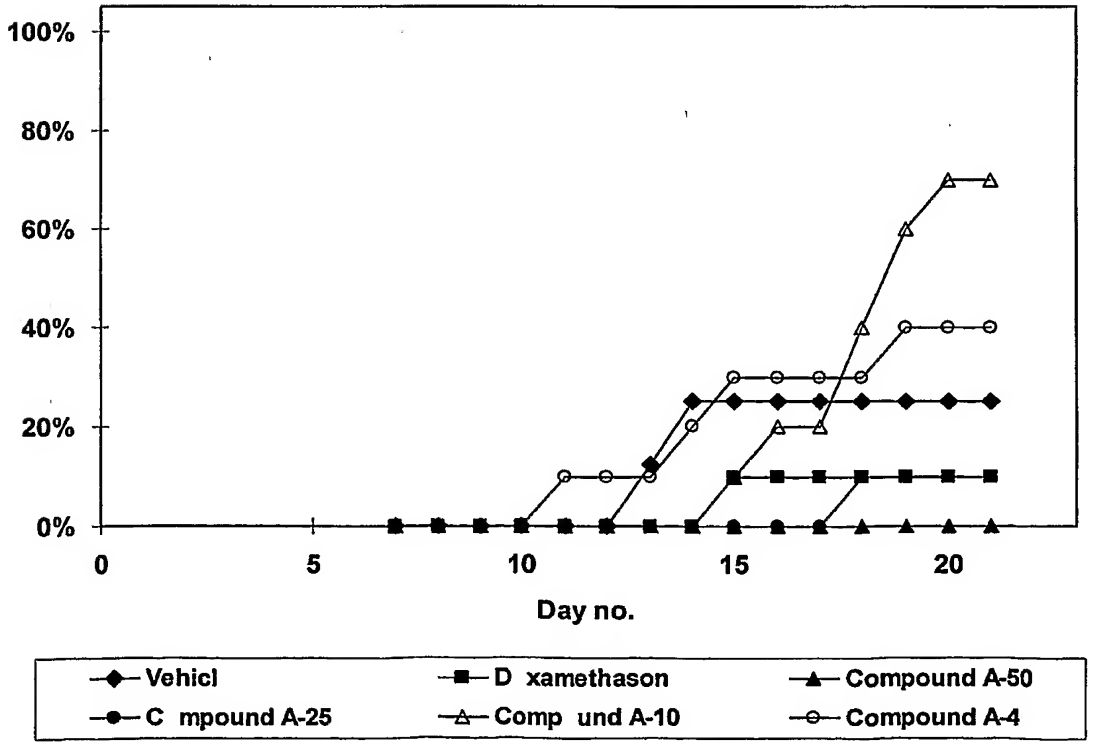
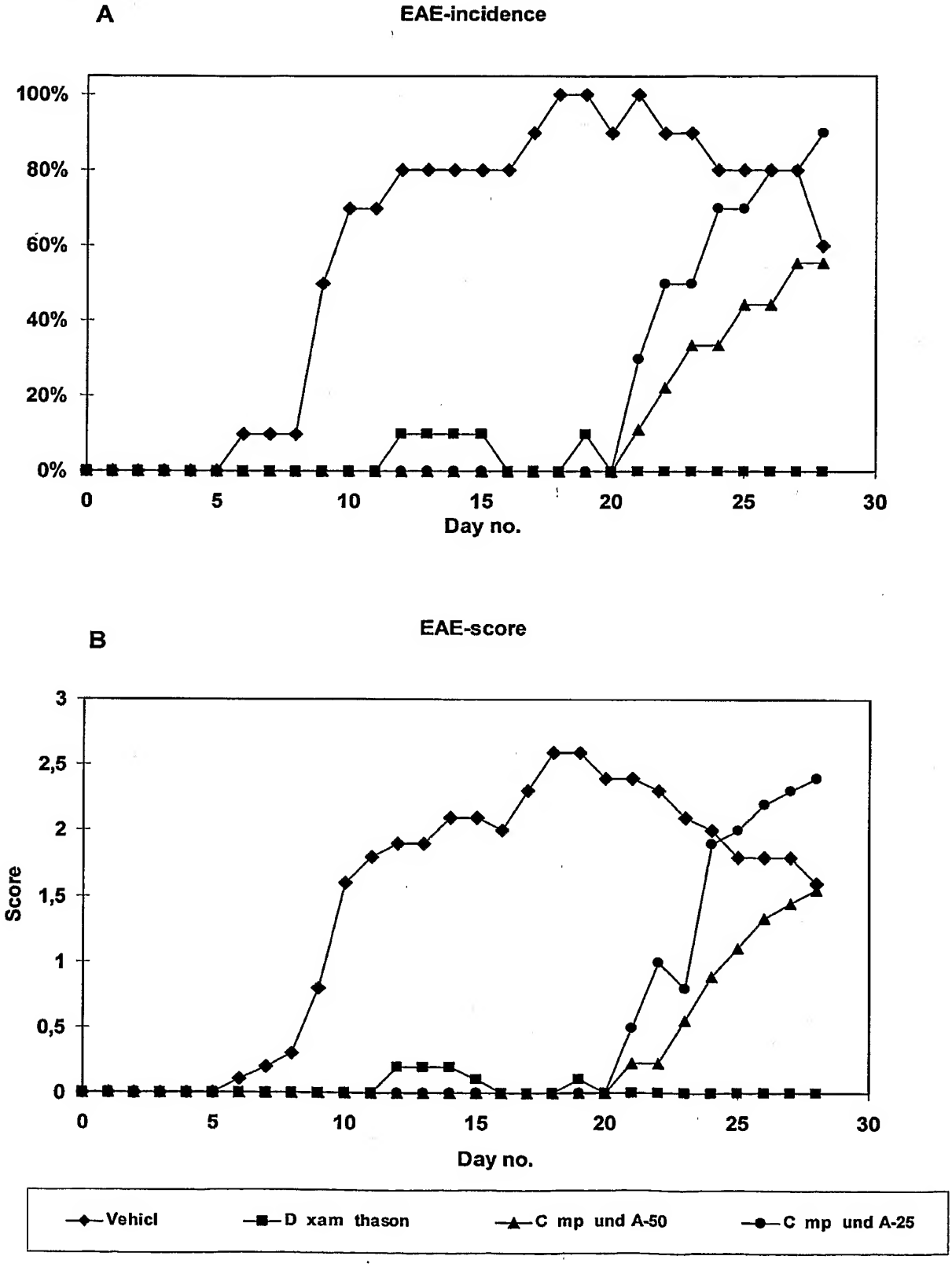


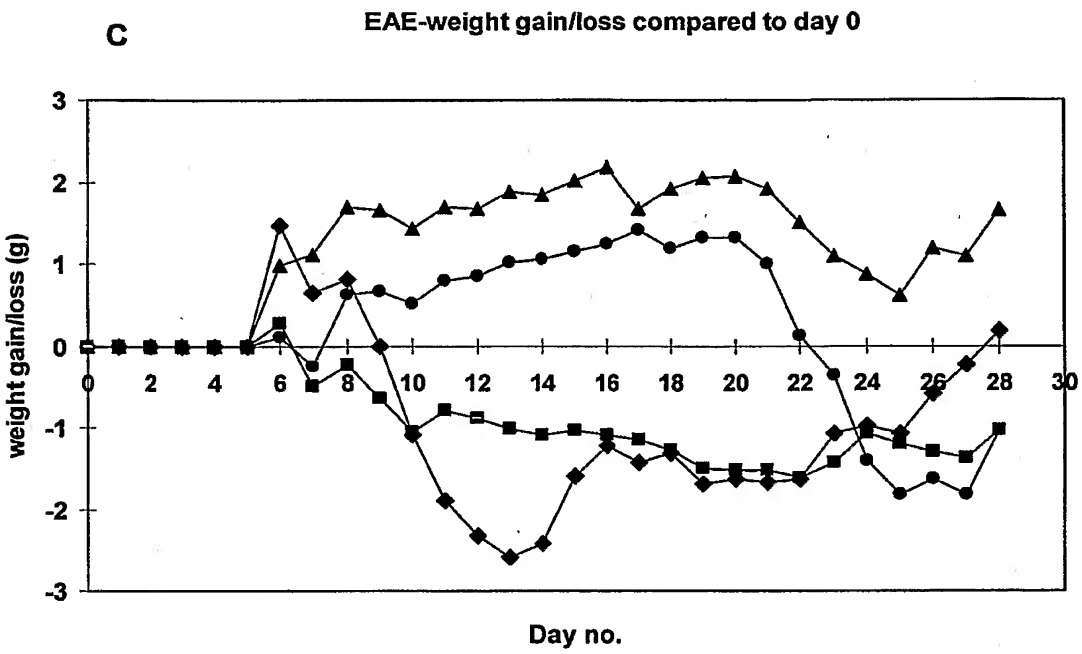
Figure 2



r

Figure 2

C



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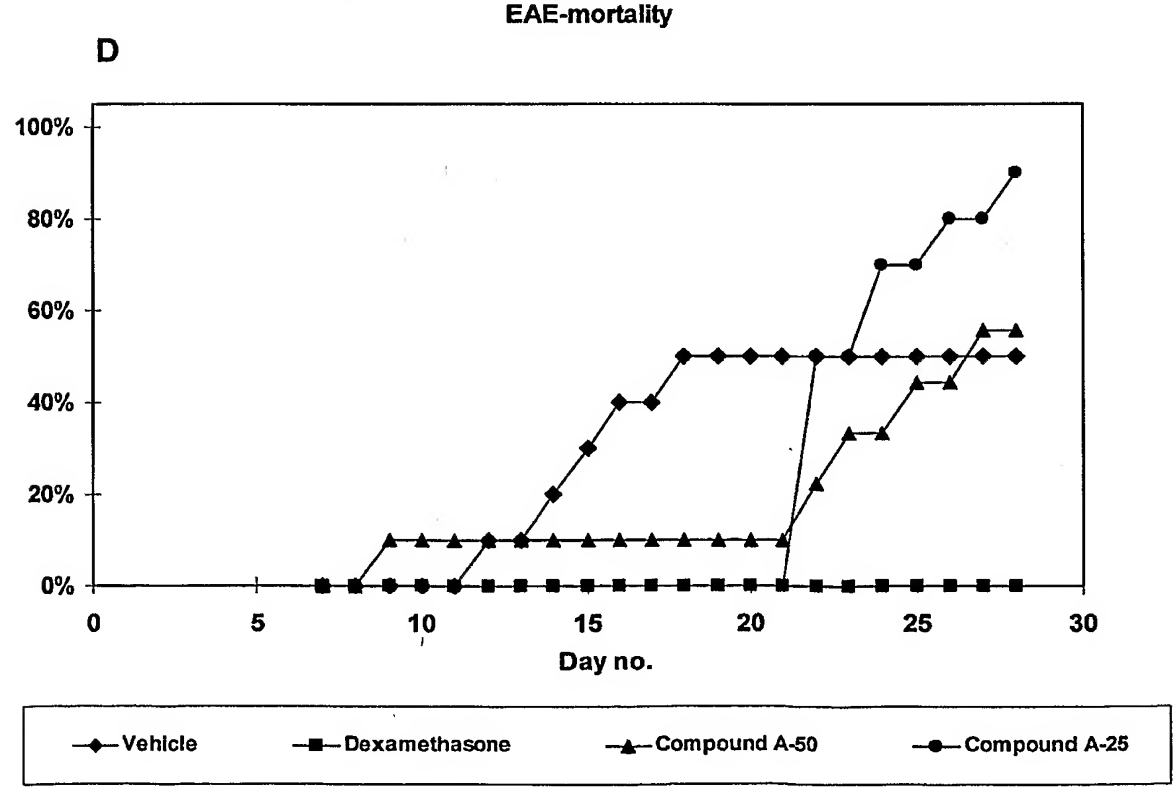
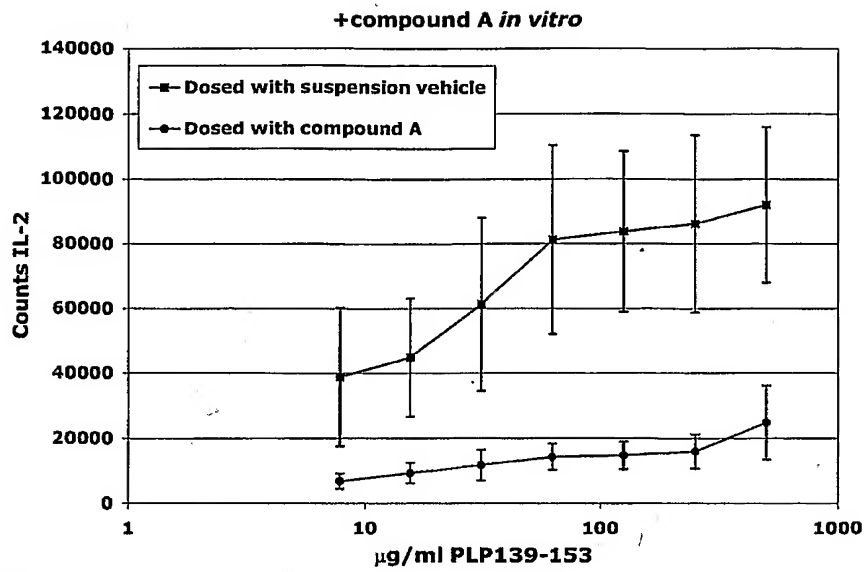


FIGURE 3

A



B

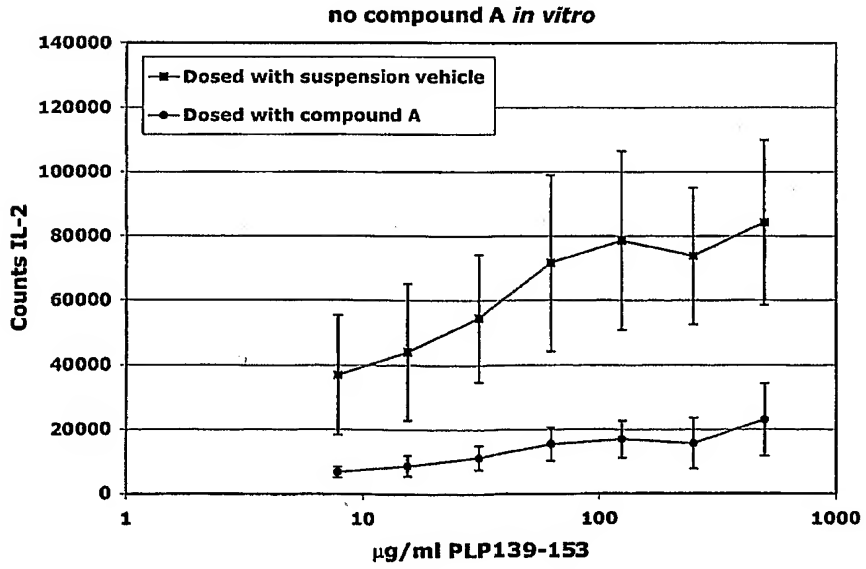
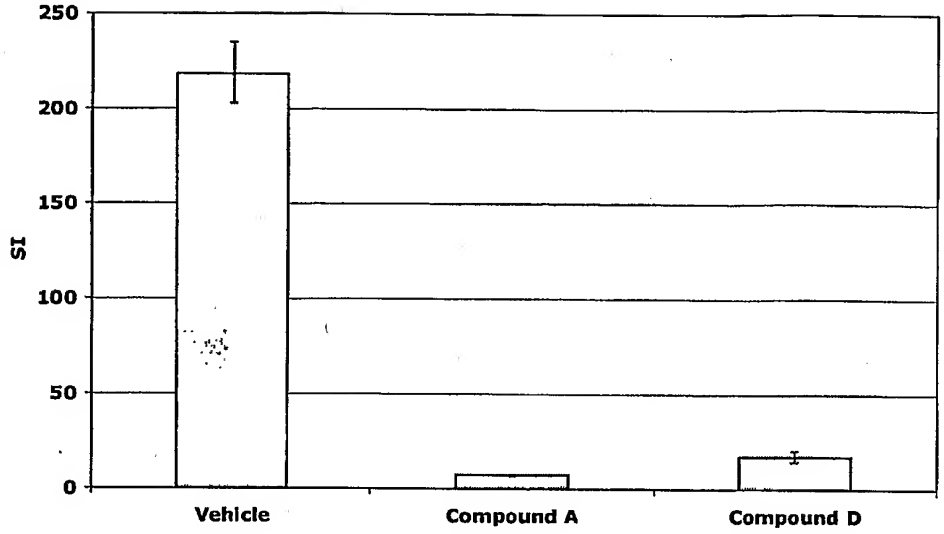
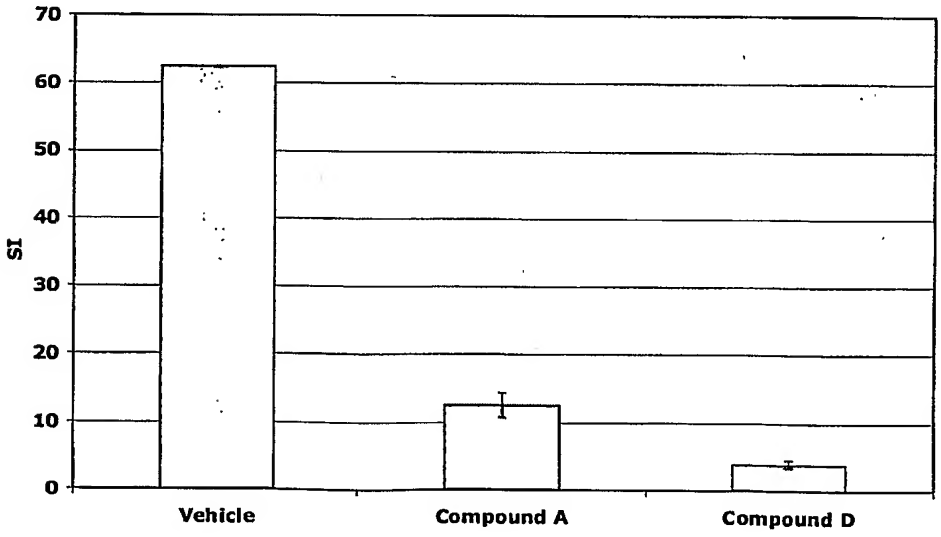


Figure 4

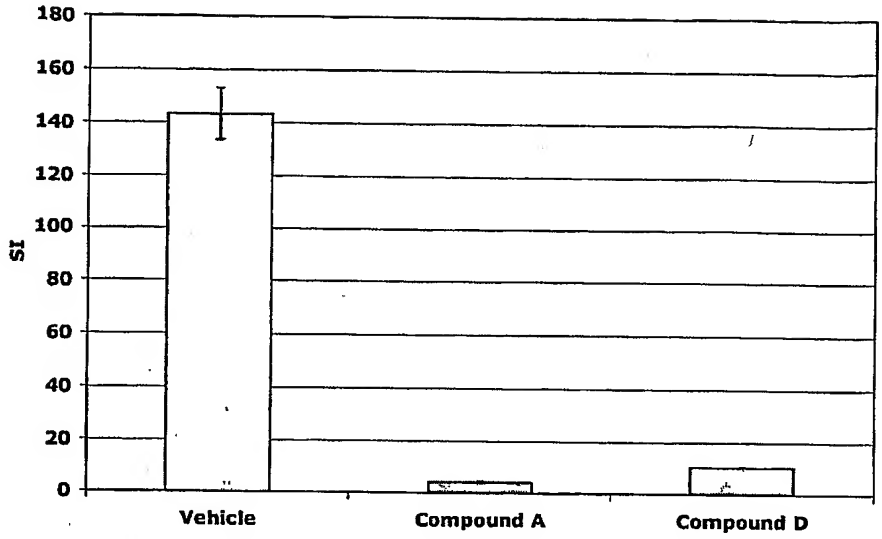
A IL-2



B IL-6



C IFN-γ



D IL-17